

CLAIMS

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1. A method of obtaining a substantially pure saponin adjuvant comprising:

(a) extracting crude saponin with methanol to obtain a methanol soluble saponin extract,

(b) purifying the methanol soluble saponin extract of step (a) by subjecting said extract to reverse phase high pressure liquid chromatography (RP-HPLC), and

(c) recovering said substantially pure saponin adjuvant.

2. A method of obtaining a substantially pure saponin adjuvants comprising:

(a) preparing an aqueous extract of crude saponin,

(b) extracting the extract of step (a) with methanol to obtain a methanol soluble extract,

(c) subjecting the methanol soluble extract of step (b) to silica adsorption chromatography to obtain fractions that have immune adjuvant activity,

(d) recovering the fractions of step (c) that contain immune adjuvant activity,

(e) purifying saponin fractions of step (d) to homogeneity by subjecting said fractions to reverse phase high pressure liquid chromatography (RP-HPLC) to obtain substantially pure saponins with immune adjuvant activity,

(f) recovering said substantially pure saponin.

1 3. Substantially pure QA-7 saponin having a retention  
2 time of approximately 9-10 minutes on RP-HPLC on a  
3 Vydac C<sub>4</sub> column having 5 µm particle size, 330 Å pore,  
4 4.6mm ID x 25 cm L in a solvent of 40mM acetic acid in  
5 methanol/water (58/42; v/v) at a flow rate of 1 ml/min.  
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7 4. Substantially pure QA-7 saponin as claimed in  
8 claim 3, wherein said saponin has immune adjuvant  
9 activity, contains about 35% carbohydrate per dry  
10 weight (assayed by anthrone), has a UV adsorption  
11 maxima of 205-210 nm, a micellar concentration of 0.06%  
12 (w/v) in water and .07% in phosphate buffered saline  
13 and causes no detectable hemolysis of sheep red blood  
14 cells at concentrations of 200 µg/ml.  
15

16 5. Substantially pure QA-7 saponin as claimed in  
17 claim 4, wherein said carbohydrate has a composition  
18 consisting of terminal rhamnose, terminal xylose,  
19 terminal glucose, terminal galactose, 3-xylose,  
20 3,4-rhamnose, 2,3-fucose, 2,3-glucuronic acid and  
21 apiose.  
22

23 6. Substantially pure QA-17 saponin having a  
24 retention time of approximately 35 minutes on RP-HPLC  
25 on a Vydac C<sub>4</sub> column having 5 µm particle size, 330 Å  
26 pore, 4.6 mm ID x 25 cm L in a solvent of 40 mM acetic  
27 acid in methanol/water (58/42; v/v) at a flow rate of  
28 1 ml/min.  
29

30 7. Substantially pure QA-17 saponin as claimed in  
31 claim 6, wherein said saponin has immune adjuvant  
32 activity, contains about 29% carbohydrate per dry  
33 weight (assayed by anthrone), has a UV adsorption

1 maxima of 205-210 nm, has a critical micellar  
2 concentration of 0.06% (w/v) in water and a 0.03% (w/v)  
3 in phosphate buffered saline, and causes hemolysis of  
4 sheep red blood cells at concentrations of 25 µg/ml or  
5 greater.

6  
7 8. Substantially pure QA-17 saponin as claimed in  
8 claim 7 wherein said carbohydrate has a composition  
9 consisting of the following monosaccharide residues:  
10 terminal rhamnose, terminal xylose, terminal galactose,  
11 terminal glucose, 2-arabinose, 2-fucose, 3-xylose,  
12 3,4-rhamnose, and 2,3-glucuronic acid and apiose.

13  
14 9. Substantially pure QA-18 saponin having a  
15 retention time of approximately 38 minutes on RP-HPLC  
16 on a Vydac C<sub>4</sub> column having 5 µm particle size, 330 Å  
17 pore, 4.6 mm ID x 25 cm L in a solvent of 40 mM acetic  
18 acid in methanol/water (58/42; v/v) at a flow rate of  
19 1 ml/min.

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21 10. Substantially pure QA-18 saponin as claimed in  
22 claim 9, wherein said saponin has immune adjuvant  
23 activity, contains about 25-26% carbohydrate per dry  
24 weight, has a UV absorption maxima of 205-210 nm, has a  
25 critical micellar concentration of .04% (w/v) in water  
26 and .02% (w/v) in phosphate buffered saline, causes  
27 hemolysis of sheep red blood cells at concentrations of  
28 25 µg/ml or greater.

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30 11. Substantially pure QA-18 saponin as claimed in  
31 claim 10, wherein said carbohydrate has a composition  
32 consisting of the following monosaccharide residues:  
33 terminal rhamnose, terminal arabinose, terminal apiose,

1 terminal xylose, terminal glucose, terminal galactose,  
2 2-fucose, 3-xylose, 3,4-rhamnose, and 2,3-glucuronic  
3 acid.

4  
5 12. A substantially pure QA-21 saponin having a  
6 retention time of approximately 51 minutes on RP-HPLC  
7 on a Vydac C<sub>4</sub> column having 5  $\mu$ m particle size, 330 Å  
8 pore, 4.6 mm ID x 25 cm L in a solvent of 40 mM acetic  
9 acid in methanol/water (58/42; v/v) at a flow rate of  
10 1 ml/min.

11  
12 13. Substantially pure QA-21 saponin as claimed in  
13 claim 12, wherein said saponin has immune adjuvant  
14 activity, contains about 22% carbohydrate per dry  
15 weight, has a UV absorption maxima of 205-210 nm, has a  
16 critical micellar concentration of about .03% (w/v) in  
17 water and .02% (w/v) in phosphate buffered saline, and  
18 causes hemolysis of sheep red blood cells at  
19 concentrations of 25  $\mu$ g/ml or greater.

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21 14. Substantially pure QA-21 saponin as claimed in  
22 claim 14, wherein said carbohydrate has a composition  
23 consisting of the following monosaccharides: terminal  
24 rhamnose, terminal arabinose, terminal apiose, terminal  
25 xylose, 4-rhamnose, terminal glucose, terminal  
26 galactose, 2-fucose, 3-xylose, 3,4-rhamnose, and  
27 2,3-glucuronic acid.

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29 15. A substantially pure saponin, other than QA-7,  
30 QA-17, QA-18, or QA-21, isolated from a crude Quillaja  
31 extract by adsorption chromatography and reverse phase

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1 chromatography by the method in Examples 3 and 4,  
2 having immune adjuvant activity and being less toxic  
3 than crude Quillaja extract.

4  
5 16. The use of substantially pure saponin in the  
6 preparation of an agent for enhancing an immune  
7 response in an individual to an antigen.

8  
9 17. A pharmaceutical composition useful for inducing  
10 the production of antibodies to an antigen in an  
11 individual comprising an immunogenically effective  
12 amount of an antigen and a substantially pure saponin  
13 wherein said amount of said substantially pure saponin  
14 is present in an amount sufficient to enhance the  
15 immune response of said individual to said antigen.

16  
17 18. A pharmaceutical composition as claimed in claim  
18 17, wherein said individual is a human or a cat.

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20 19. A pharmaceutical composition as claimed in claim  
21 17 or 18, wherein said antigen is a gp70-containing  
22 protein.

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24 20. A pharmaceutical composition as claimed in claim  
25 17, 18 or 19 wherein said saponin is QA-7, QA-17, QA-18  
26 or QA-21.

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28 21. A pharmaceutical composition as claimed in claim  
29 17, 18 or 19, wherein said saponin is a mixture of two  
30 or more of the purified saponins QA-7, QA-17, QA-18 or  
31 QA-21 or any of the saponins described in claim 15.

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1 22. A pharmaceutical composition as claimed in claim  
2 17, 18 or 19, wherein said saponin is a component other  
3 than QA-7, QA-17, QA-18 or QA-21, but is isolated from  
4 a crude Quillaja saponin mixture, possesses immune  
5 adjuvant activity, and is substantially purified by  
6 adsorption chromatography and reverse-phase  
7 chromatography as outlined in Examples 3 and 4, and is  
8 less toxic than crude Quillaja saponin mixtures.

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10 23. A pharmaceutical composition as claimed in claim  
11 17, 18 or 19, wherein said saponin is substantially  
12 free of toxic component QA-19.

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